# Shelf-life of Bacillus subtilis B298 inclusion in biopesticide microencapsule formula (BMF) and its efficacy in supressingchili anthracnose

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# Shelf-life of *Bacillus subtilis* B298 inclusion in biopesticide microencapsule formula (BMF) and its efficacy in supressingchili anthracnose

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**Abstract**. *Bacillus subtilis* B298 is an antagonistic bacterium isolated from the rhizosphere of potatoes;its capable in controlling diseases both caused by fungi and bacteria. Inclusion of *B. subtilis* B298 inBMF is an effort to extend its shelf life, which maintain its stability and relatively more resistant to environmental change conditions. This study was aimed to determine the shelf life of *B. subtilis* B298 in BMF, the effectiveness of *B. subtilis* B298 in BMF to suppress chili anthracnose disease. The study was experimental with the application of *B. subtilis* B298 in BMF by chili seed coating for 24 hours then seed sowing, and followed by watering the biopesticide around the roots of plants aged 10, 20 and 30 days after planting. The study used a Completely Randomized Block Design with four treatments (control, *B. subtilis* B298 BMF, fungicide and combination of *B. subtilis* B298 BMF and fungicide), the treatment was repeated six times. Variables observed were; the population of *B. subtilis* B298 in the BMF, the disease intensity and the rate of infection in anthracnose by *Colletotrichum* sp. Results of the study showed that *B. subtilis* B298 in biopesticide microencapsule formula was capable to survive for 5 weeks with a population of 14.4 $10^7$ cfu /g and its treatment revealed suppress chili anthracnose by 53.71%.

*Key words*: *Bacillus subtilis* B298, shelf life, microencapsule, chili anthracnose

## 1. Introduction

A *Bacillus* is a genus of bacteria that has abundant in the rhizosphere, has a role as a driver of plant growth and agents in controlling plant pathogens. *Bacillus subtilis* is a bacterium that can be found in soil, plants, water and air. According to [1] this bacterial strain shows several mechanisms involved in its role as a plant growth promoter (PGPR = plant growth promoting rhizobacteria), which is shown by producing metabolite activity as an IAA producer, a phosphate solvent, a siderophore producer.

Furthermore, it was stated that *Bacillus* as a control pathogen by producing antibiotics, lipopeptides and hydrolytic enzymes. The activity of these bacteria has an influence on pathogens and plant growth. *B. subtilis* potato isolates are capable to produce siderophores, phosphate solvents and IAA-producer and are capable to control bacterial wilt disease in chili [2,3]. Naturally, *Bacillus* sp. colonize the roots, can be stable in contact with plants and settle as a rhizosphere microbe that can stimulate plant growth; therefore it can be function as a biofertilizer [4,5].

In addition, as plant pathogens antagonists bacteria and plant growth boosters; it also can increase plant resistance or induce resistance (Induced Systemic Resistance). Five isolates of *B. subtilis* (B46, B 209, B211, B298, and B315) derived from the potato rhizosphere were capable to control the bacterial wilt disease of potatoes [6]. It has been reported that the bacterial is safe for plants and can even increase plant growth and resistance because it produces siderophores, IAA, as a phosphate solvent and produces antimicrobial compounds such as hydrolitic enzyme chitinase, protease, and amylase [7,8,9]. *B. subtilis* B298 is capable to control bacterial wilt disease and chili anthracnose with suppressive effectiveness of 74.66% and 53.94% with a liquid formula [2].

The formulation of *B. subtilis* B298 microencapsulant biopesticide was made with the aim to make it more stable, more viable, have better durability and effectiveness in controlling pathogens, more practical in transportation and application. It has been reported that inclusion of *B. subtilis B298* in microencapsulation biopesticide formula induced plant systemic disease resistance on chili as total phenol of the treated plant increased [10]. that Economically, the microencapsulation formula is more efficient and environmentally friendly. Microencapsulation formula shows higher viability compared to liquid formula [11].

Anthracnose disease is a major disease in chilli that is still difficult to control because the pathogen is an airborne and has several species. In Indonesia, losses from chili due to anthracnose disease can reach 50-100% [12]. The disease development is quite fast because of its spread through the air, as average temperature and humidity Indonesia is suitable for the development of anthracnose disease. Control of anthracnose disease carried out so far is by synthetic fungicides which, if applied continuously and unwise, can have a negative impact on the environment. The use of biological agents *B. subtilis* B298 in the microencapsulant formula (*B. subtilis* B298 in BMF) is expected to suppress the development of this disease.

The objectives of the study were to: (1) determine the shelf life of *B. subtilis* B298 in BMF, (2) determine the effectiveness of *B. subtilis* B298 in BMF in suppressing anthracnose chili disease.

## 2. Research Methods

### *2.1. Shelf-life test of B. subtilis* B298 *in BMF*

Microencapsulant material called encapsulant consists of maltodextrin and arabic-gum with a ratio of 1: 1, 2: 3 and 3: 2, then water was added to form paste. Each microencapsulant formula weighing 50 g plus 30 mL water then sterilized at 121ºC and pressure 15 psi for 25 min. *B. Subtilis* B298 was grown on liquid Yeast-peptone medium, shaked at room temperature for 2x24 h. Suspension of *B. subtilis*B298 as much as 0.1 v / w with density x.$10^8$cfu / mL was added to the microencapsulant material, then dried using a *benchtop K* type *freeze-drier* at - 73ºC, for 14 h. The results of this drying are then blended until smooth and stored as a microencapsulant formula (*B. subtilis* B298 in BMF).

Variables observed: viability of *B. subtilis* population observed every week for 5 weeks. The *B. subtilis* viability evaluation in the microencapsulant formula was conducted by dissolving 1g of the formula in 9 mL of sterile water and a series of dilution with 0.85% physiological NaCl. Until dilutions of 6,7 and 8, 10 µL were grown on the YPGA medium by drop plate method [13]. Viability testing also included confirmation of Gram's properties with 3% KOH and the ability to produce amylase and chitinase enzymes, using starch hydrolysis medium and medium containing colloidal chitin [14].

### *2.2. Application of B. subtilis* B298 *in* BMF *to control anthracnose dissease on chili*

Application on red chili plants to control anthracnose disease, with treatments arranged by completely randomized block design consisting of 4 treatments with 6 replications, as follows

K: control (without biopesticide formula)

B: application of *B. subtilis* B298 in BMF

F: application of fungicides with active ingredients Carbendazim

BF: application of a combination of *B. subtilis* B298 in BMF and fungicide

This experiment was carried out at lowlands 110 and 400 m above sea level. The *B. subtilis* B298 in BMF used was the best formula from the previous established, that is formulation in maltodextrin: arabic gum = 3: 2. The application of this biopesticide is to cover the seeds, and when the plants are 10.20 and 30 dd, they are poured on the soil around the plants with a concentration of 2g formula / L water. The variables observed were incubation period (IP), the intensity of anthracnose disease. For anthracnose disease using the formula according to [15] as follows;

$$IP = \frac{\Sigma\, n.v}{Z.\, N} \times 100\%$$

with damage score values based on symptoms in the fruit (anthracnose) and leaves (leaf spot) as follows: ***0***: no damage, ***1***: damage area on fruit and or leaves > 0 - <10%, ***2***: damage area > 10 - <20%, ***3***: damage area > 20 - <40%, ***4:*** damage area :> 40 - <60%, and ***5*** :damage area > 60% (modification of [16]).

Infection rate was calculated using the van der Plank formula [17] with the type of disease development of "*compound interest disease*" **r** = 2.3 / t2-t1 [log 1 / (1-X2) - log 1 / (1-X1)] unit / day. Effectiveness of disease suppression is calculated based on comparison of control disease intensity and treatment disease intensity according to [18], as follows;

$$\text{Effectivity} = \frac{(\text{IP control} - \text{IP Treatment})}{\text{IP control}} \times 100\ \%$$

## 3. Results

### *3.1. Shelf life test of B. subtilis B298 in BMF*

Results of the viability test of *B. subtilis* B298 in BMF were seen from the colonies on theYPGA medium isolated from the formula every week by calculating the population in total plate counting (TPC). The population of *B. subtilis* B298 in BMF can be seen in Figure 1.

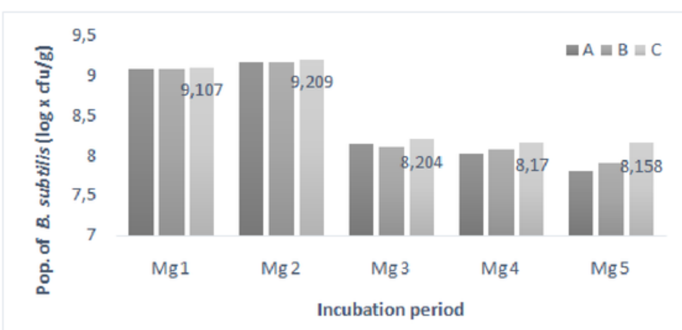


Figure 1. *B. subtilis* B298 in BMF population in 5 weeks incubation time
Note : **A**: M(maltodekstrin):GA(Gom Arab)= 1:1; **B**: M:GA= 2:3; **C**: M:GA= 3:2

Based on Figure 1, the best population is the 3rd (M: GA = 3: 2) with a population of 14.4. 107 cfu / g formula. B. subtilis B298 can still survive the shelf life at BMF for up to 5 weeks

*3.2. Evaluation of B. subtilis B298 in BMF application on chili anthracnose disease*

The main disease of chili is anthracnose by the fungus *Colletotrichum* sp. The intensity of the antacnosis disease at two altitudes is shown in Table 1.

Table 1. Incubation period (Ip), disease intensity (DI) and effectiveness of disease control in Tambaksari Kidul Village (110 m asl) and Gandatapa (400 m asl)

| Treatment | 'Tambaksari Kidul' village | | | 'Gandatapa' village | | |
|---|---|---|---|---|---|---|
| | Ip, day after planting | DI (%) | Effectivity (%) | Ip, day after planting | DI (%) | Effectivity (%) |
| K | 55 | 22,5 **a** | - | 58 | 29,17 **a** | - |
| B | 62 | 10,83 **b** | 51,87 | 65 | 13,50 **b** | 53,71 |
| F | 62 | 18,33 **a** | 18,53 | 66 | 20 **ab** | 31,44 |
| BF | 64 | 14.17 **b** | 37,02 | 66 | 11,67 **b** | 59,99 |

Note : **K**: control; **B**: *B. subtilis* B298 in BMF; **F**: fungicide with active ingredient of carbendazyme; **BF** combination of *B. subtilis* B298 in BMF and fungicide. Figures followed by the same letters in the same column show no significant difference in the 5% level LSD

The effectiveness of anthracnose disease control in the combination treatments of *B. subtilis* B298 in BMF and fungicide formula at two altitudes was not significantly different from the treatment of *B. subtilis* B298 in BMF alone. In Gandatapa (400 m asl) shows the effectiveness of the control up to 53.71%. The development of anthracnose disease in the Tambaksari Kidul and Gandatapa villages for 5 observations up to 83 days afterwards is shown in Figure 2. The pattern of disease development at both altitudes was relatively the same, with the highest control and the average speed of disease development is 0.024 units / day.

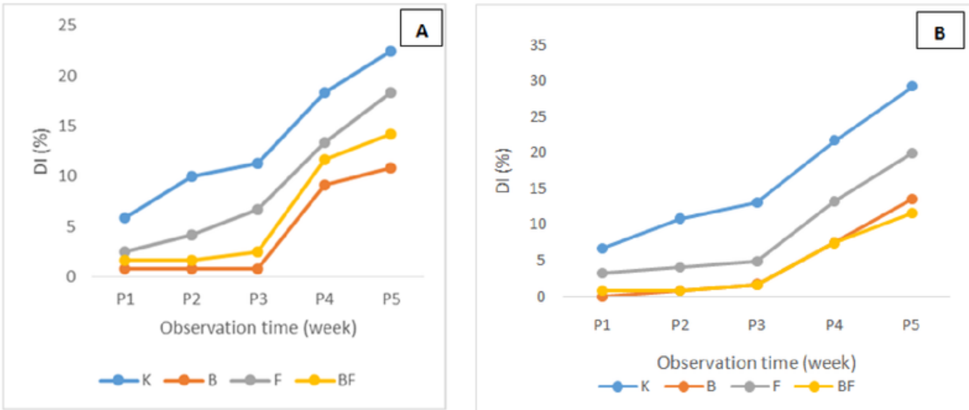


Figure 2. Anthracnose disease development at two height level of A:110 and B: 400 m asl (above sea level)

The yields differences in in these two places is due to weather relative differences on the temperature and humidity, in Gandatapa with a height of 400 m above sea level, plants are greener and survive longer, therefore the harvest period is longer. According to [19] that anthracnose disease develops well under optimal conditions, namely temperature 27ºC and humidity 80%.

## 4. Discussion

In order to protect an active ingredient can be done by coating through microencapsulation process in which the liquid active ingredient processed into very small particle sizes in the range 1-5000 µm. By microencapsulated, the active ingredient can be distributed evenly and extends to the location of the target application. Inclusion an active inggredient in microencapsulation resulted that it is protected from the environment, separated from incompatible components, protected and maintained in the formulation, finally it is released slowly (slow release) and long and ongoing [20].

Microencapsulant biopesticide formula (MBF) is an innovative biopesticide formulation. *B. subtilis* B298 in BMF is more durable, more practical in storage and transportation, as [11] has been stated that the microencapsulant formula is more stable, and *B. subtilis* as an active ingredient has a longer and more effective viability compared to the liquid formula.

As a conclusion, this study resulted that *B. subtilis* B298 in BMF has a shelf life of up to 5 weeks after the formulation with a population of 14.4. $10^7$ cfu / g formula. BMF shows the effectiveness of controlling anthracnose chili by 53.71%. Further studies needed are an evaluation of the presence and viability of *B. subtilis* B298 after the crop is harvested, to support sustainable agriculture.

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